Discussion

Tamoxifen performs its anti-tumor activity via many pathways, both ER-dependent and ER-independent. In the present study, we attempted to identify novel anti-tumor compounds among RIDs that were designed from a lead compound, tamoxifen. We synthesized forty-eight RIDs by efficient methods including the three-component coupling reaction [9]. To screen them for promising anti-tumor compounds, we used the JFCR39 panel and an ER binding assay. By this means, we found two RIDs, RID-SB1 and RID-SB8, that showed stronger growth-inhibitory activity than tamoxifen and are expected to have unique modes of action.

The forty-eight RIDs showed growth-inhibitory activities against JFCR39 over a wide range of MG-MIDs from 0.85 to 43.7 μM. Forty out of forty-eight RIDs (83%) showed higher growth-inhibitory activity than tamoxifen (MG-MID = 7.41 μM). The SAR study indicated that the structures of aminoalkoxyphenyl groups at the C-1 position and the common central ethylenic double bond were important in retaining a high level of growth-inhibitory activity.

The ERα binding activity of the RIDs was spread across a huge range of IC_{50} values from 26.7 to > 10000 nM. In general, the 1st-generation RIDs exhibited ERα binding activities. Among them RID-G showed the most potent activity (IC_{50} = 26.6 nM), which is close to the activity of tamoxifen (IC_{50} = 21.1 nM). In contrast, a number of RIDs in the 2nd-generation were inactive with respect to ERα binding. The SAR study clearly indicated that both the phenyl and ethyl groups at the C-2 position of the central ethylenic bond were essential for ERα binding activity. In addition, there was a tendency for RIDs bearing more bulky structures around the double bond (RID-SB7 to SB12 and SG7 to SG12) to display higher ERα binding activity.

According to the above observations, the forty-eight RID compounds were profiled by growth-inhibitory activity and ERα binding activity (Fig. 5). Interestingly, these two activities didn't correlate in the forty-eight RIDs. Some of them, such as RID-G, showed high levels in both activities, while others were active in only one. In the latter group, we focused on RID-SB1 and RID-SB8 because they were highly active in growth-inhibition but were inactive in regard to ERα binding. It was suggested that they inhibited the cell growth via an ER-independent mechanism and thus may inhibit the growth of cancer cells by a different mode of action than tamoxifen. The COMPARE analysis was therefore performed on this study, and it revealed that both RID-SB1 and RID-SB8 showed very weak correlation coefficients ($r < 0.4$) with tamoxifen. These results support the hypothesis above.

So far, a number of tamoxifen derivatives have been designed and synthesized with the aim of enhancing ER binding activity and reducing side-effects. However, this has not been entirely successful. For example, clomifene [24], ospemifene [25, 26], iodoxifene [27], raloxifene [27], arzoxifene [28], lasofoxifene [29] and levormeloxifene [30, 31], possess high ER binding activity, while still having such disadvantages as biotransformation effect, biological isomerization, thromboembolic effect and agonist action in uterus [32]. Most of these tamoxifen derivatives show very similar effects as tamoxifen and have been proposed considering only the effect on ER. In the present study, RID-SB1 and RID-SB8, while having completely lost ER binding activity, display approximately 5-fold higher antitumor activity than tamoxifen, indicating a non-ER target in its anti-tumor process. Such non-ER function might be useful in unveiling new targets to inhibit tumor cell proliferation. Therefore, RID-SB1 and RID-SB8 maybe novel drug candidates for breast cancer but also for other malignancies.

We finally attempted to predict the action modes of RID-SB1 and RID-SB8 by using our JFCR39 drug database and COMPARE analysis. The two RIDs did not correlate with any currently available anti-cancer drug, suggesting that they could be examples of a novel class of anti-cancer drug. The COMPARE analysis suggested some targets shown in Table 2, however, this needs to be verified by biological testing in the future.

In conclusion, we synthesized forty-eight tamoxifen-derivatives, RIDs, and screened them, searching for novel candidates of a new class of anti-cancer drug. We identified RID-SB1 and RID-SB8 as having potent tumor growth-inhibitory activity but having completely lost ERα binding activity. Based on these results and COMPARE analysis, it was suggested that RID-SB1 and RID-SB8 had unique action modes, different from those of current anti-cancer drugs including tamoxifen. RID-SB1 and RID-SB8 merit further investigation.

Acknowledgment

We thank Dr. R. H. Shoemaker and the late Dr. K. D. Paull for the establishment of JFCR39 and COMPARE analysis, and also thank Ms. Y. Nishimura, Ms. Y. Ohashi, Ms. M. Okamura, Ms. M. Seki, Ms. Y. Mukai and Ms. N. Tamaki for technical assistance.

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Figure legends

Fig. 1. Chemical Structures of tamoxifen, 4-hydroxytamoxifen and RIDs. (A) tamoxifen, 4-hydroxytamoxifen and 1st-generation RIDs. (B) 2nd-generation RIDs. (C) 3rd-generation RIDs.

Fig. 2. Dose response curves of (A) tamoxifen and (B) RID-SB1 against growth of JFCR39 cancer cell lines. The growth inhibition of tamoxifen and RID-SB1 on 39 cell lines (Br, breast; CNS, central nervous system; Co, colorectal; Lu, lung; Me, melanoma; Ov, ovarian; Re, renal; St, stomach; xPg, prostate) were measured as described in Materials and methods.

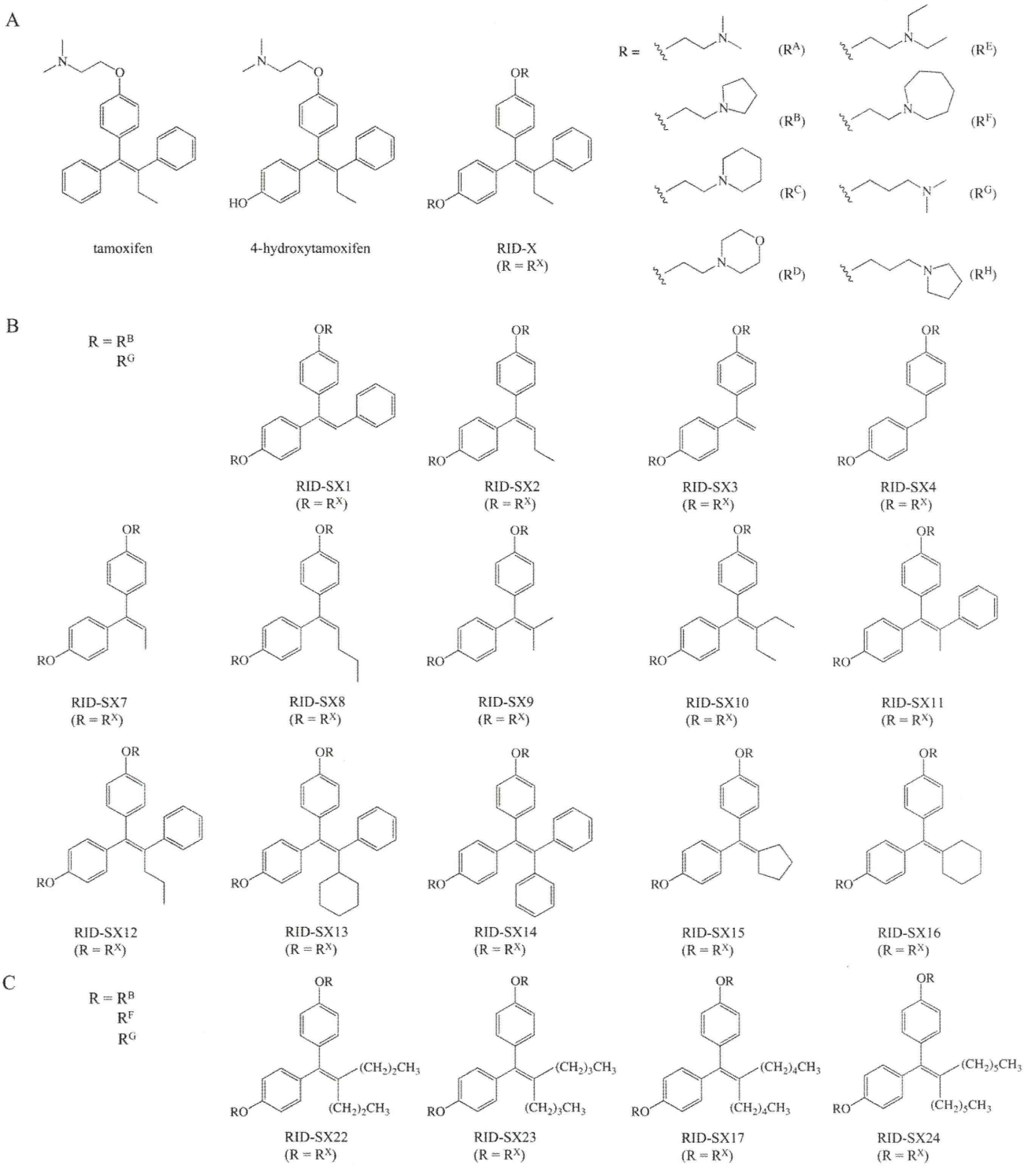
Fig. 3. Fingerprints of tamoxifen, RID-SB1 and RID-SB8. Fingerprint shows the differential growth inhibition pattern of chemicals against JFCR 39 cancer cell lines. The *X*-axis represents difference in logarithmic scale between mean of $LogGI_{50}$ values for 39 cell lines and the $LogGI_{50}$ for each cell line. Bars to the right of 0 indicates cell lines that are sensitive to the compound, in contrast, bars on the left of 0 means the resistance. MG-MID, mean of $LogGI_{50}$ values for 39 cell lines; Delta, difference between the MG-MID and the Log GI_{50} value for the most sensitive cell line; Range, difference between the $LogGI_{50}$ values for the most resistant cell and the most sensitive cell line.

Fig. 4. Competitive binding curves of estradiol, tamoxifen, 4-hydroxytamoxifen, RID-SB1 and RID-G. ERα competitive binding activity was measured as described in Materials and methods. Estradiol, tamoxifen, 4-hydroxytamoxifen, RID-G and RID-SB1 are represented as ○, ◇, □, ■, ▲ respectively.

Fig. 5. The scattergram of RIDs for the proliferation inhibition activity and ERα binding activity. The *X*-axis (-MG-MID) represents the proliferation inhibition of forty-eight RIDs in 39 cell lines. The *Y*-axis (-Log (IC_{50})) represents the competitive ERα binding

1 activity. Tamoxifen, RID-SB1and SB8 are represented as ◇, □, △, respectively.

Fig. 1


A
tamoxifen
4-hydroxytamoxifen
RID-X
(R = R^X)
R =
(R^A)
(R^B)
(R^C)
(R^D)
(R^E)
(R^F)
(R^G)
(R^H)
B
R = R^B
R^G
RID-SX1
(R = R^X)
RID-SX2
(R = R^X)
RID-SX3
(R = R^X)
RID-SX4
(R = R^X)
RID-SX7
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RID-SX13
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RID-SX16
(R = R^X)
C
R = R^B
R^F
R^G
RID-SX22
(R = R^X)
RID-SX23
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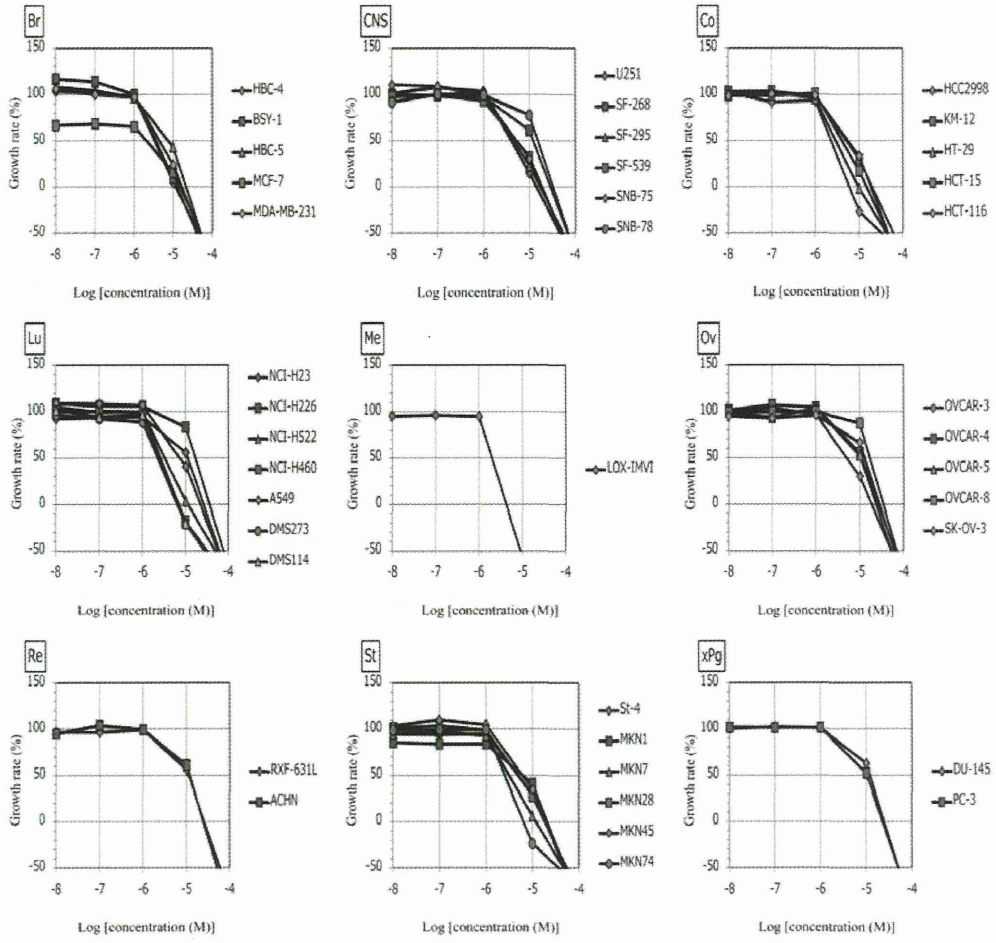
RID-SX24
(R = R^X)

Fig. 2A

A


Br
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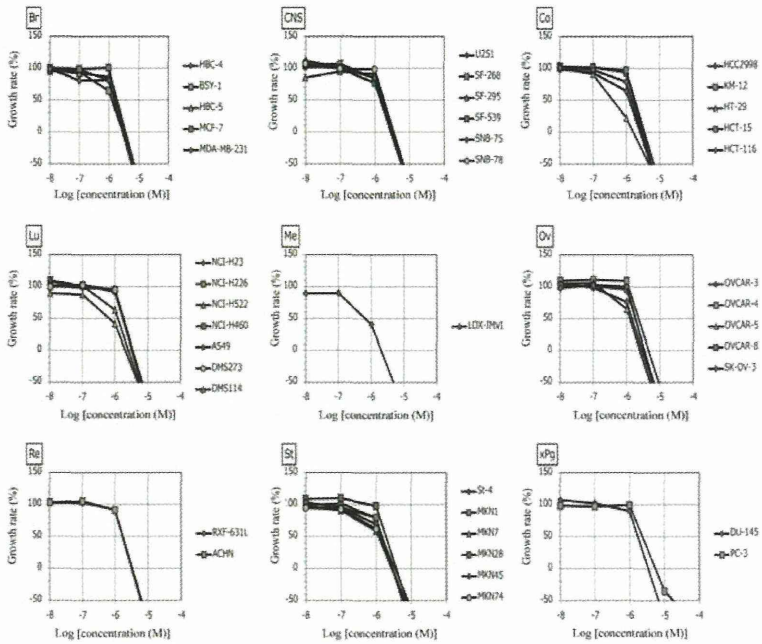
BSY-1
HBC-5
MCF-7
MDA-MB-231
CNS
U251
SF-268
SF-295
SF-539
SNB-75
SNB-78
Co
HCC2998
KM-12
HT-29
HCT-15
HCT-116
Lu
NCI-H23
NCI-H226
NCI-H522
NCI-H460
A549
DMS273
DMS114
Me
LOX-IMVI
Ov
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3
Re
RXF-631L
ACHN
St
St-4
MKN1
MKN7
MKN28
MKN45
MKN74
xPg
DU-145
PC-3
Growth rate (%)
Log [concentration (M)]

Fig. 2B

B


Br
Growth rate (%)
Log [concentration (M)]
HBC-4
BSY-1
HBC-5
MCF-7
MDA-MB-231
CNS
U251
SF-268
SF-295
SF-539
SNB-75
SNB-78
Co
HCC2998
KM-12
HT-29
HCT-15
HCT-116
Lu
NCI-H23
NCI-H226
NCI-H522
NCI-H460
A549
DMS273
DMS114
Me
LOX-IMVI
Ov
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3
Re
RXF-631L
ACHN
St
St-4
MKN1
MKN7
MKN28
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MKN74
xPg
DU-145
PC-3

Fig. 3

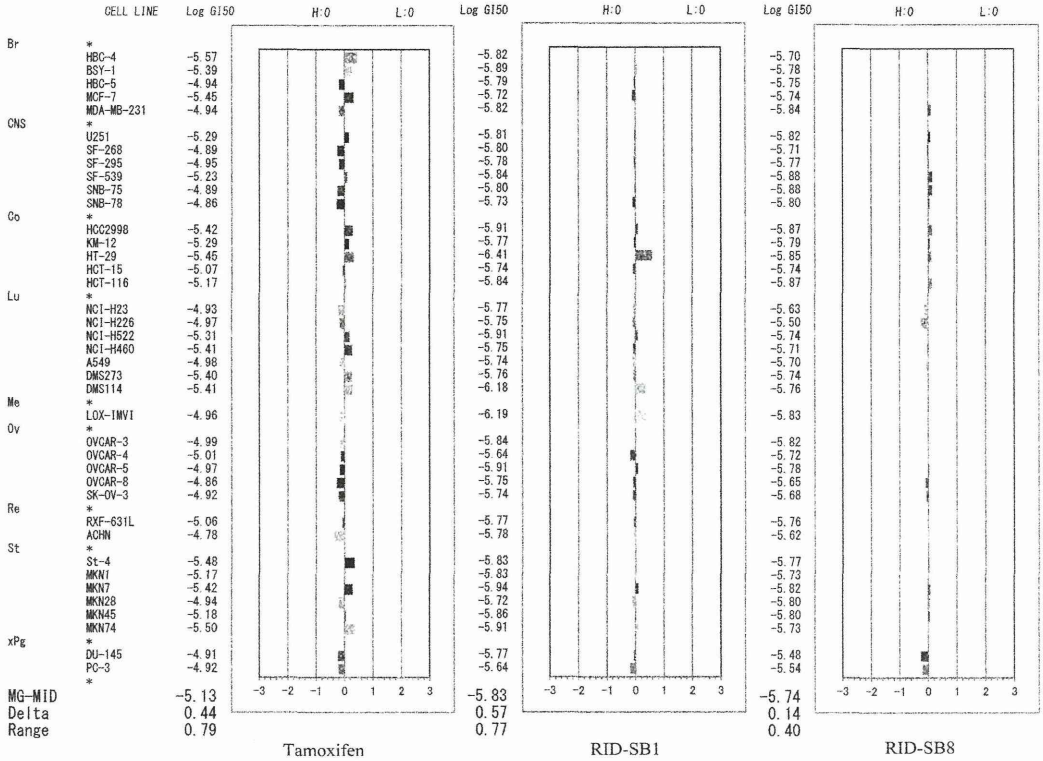
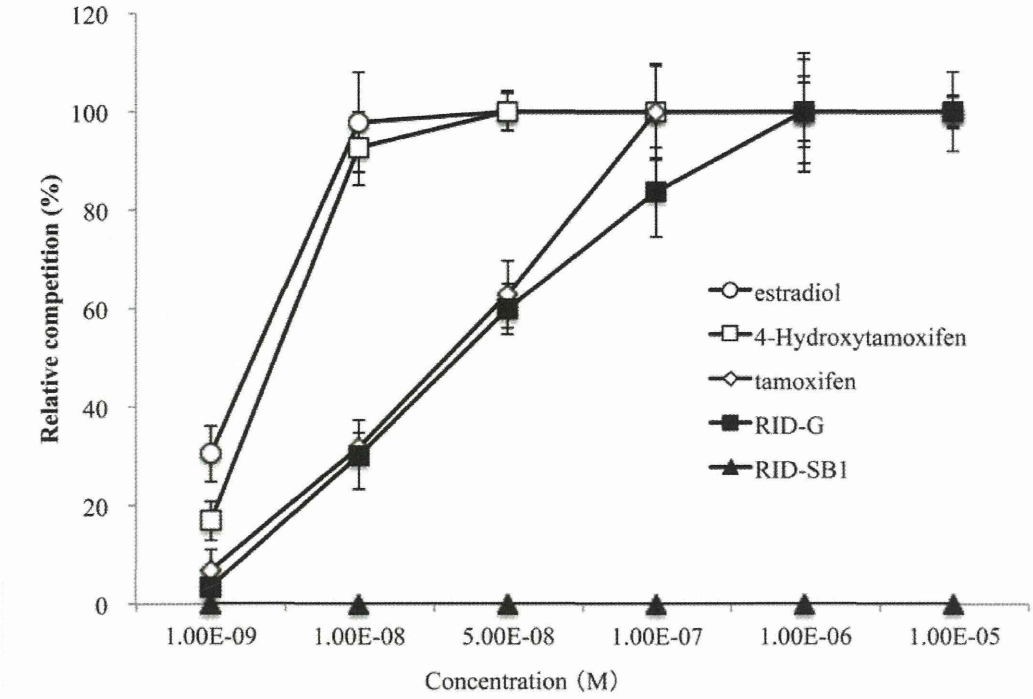

CELL LINE
Log GI50
H:0
L:0
Br
HBC-4 -5.57 -5.82 -5.70
BSY-1 -5.39 -5.89 -5.78
HBC-5 -4.94 -5.79 -5.75
MCF-7 -5.45 -5.72 -5.74
MDA-MB-231 -4.94 -5.82 -5.84
CNS
U251 -5.29 -5.81 -5.82
SF-268 -4.89 -5.80 -5.71
SF-295 -4.95 -5.78 -5.77
SF-539 -5.23 -5.84 -5.88
SNB-75 -4.89 -5.80 -5.88
SNB-78 -4.86 -5.73 -5.80
Co
HCC2998 -5.42 -5.91 -5.87
KM-12 -5.29 -5.77 -5.79
HT-29 -5.45 -6.41 -5.85
HCT-15 -5.07 -5.74 -5.74
HCT-116 -5.17 -5.84 -5.87
Lu
NCI-H23 -4.93 -5.77 -5.63
NCI-H226 -4.97 -5.75 -5.50
NCI-H522 -5.31 -5.91 -5.74
NCI-H460 -5.41 -5.75 -5.71
A549 -4.98 -5.74 -5.70
DMS273 -5.40 -5.76 -5.74
DMS114 -5.41 -6.18 -5.76
Me
LOX-IMVI -4.96 -6.19 -5.83
Ov
OVCAR-3 -4.99 -5.84 -5.82
OVCAR-4 -5.01 -5.64 -5.72
OVCAR-5 -4.97 -5.91 -5.78
OVCAR-8 -4.86 -5.75 -5.65
SK-OV-3 -4.92 -5.74 -5.68
Re
RXF-631L -5.06 -5.77 -5.76
ACHN -4.78 -5.78 -5.62
St
St-4 -5.48 -5.83 -5.77
MKN1 -5.17 -5.83 -5.73
MKN7 -5.42 -5.94 -5.82
MKN28 -4.94 -5.72 -5.80
MKN45 -5.18 -5.86 -5.80
MKN74 -5.50 -5.91 -5.73
xPg
DU-145 -4.91 -5.77 -5.48
PC-3 -4.92 -5.64 -5.54
MG-MID -5.13 -5.83 -5.74
Delta 0.44 0.57 0.14
Range 0.79 0.77 0.40
-3 -2 -1 0 1 2 3
Tamoxifen
RID-SB1
RID-SB8

Fig. 4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100
80
60
40
20
0
Relative competition (%)
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1.00E-08
5.00E-08
1.00E-07
1.00E-06
1.00E-05
Concentration (M)
estradiol
4-Hydroxytamoxifen
tamoxifen
RID-G
RID-SB1

Fig. 5

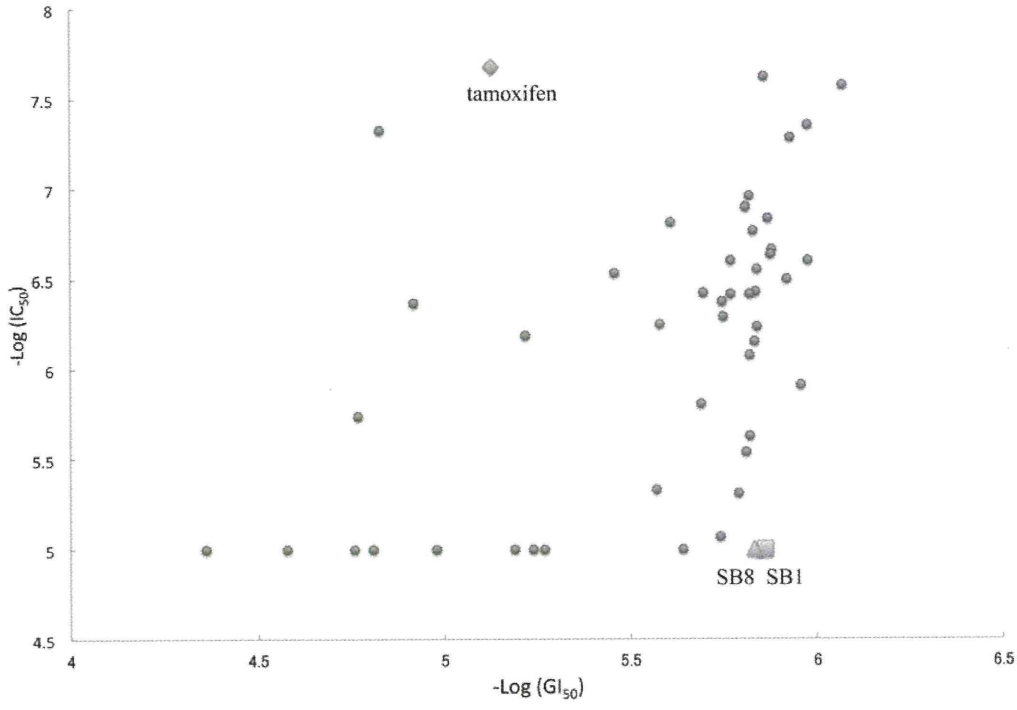
8
7.5
7
6.5
6
5.5
5
4.5
-Log (IC50)
tamoxifen
SB8
SB1
4
4.5
5
5.5
6
6.5
-Log (GI50)

Table 1. Anti-tumor activity and ERα binding capacity of RIDs

Chemical	MG-MID (μM)	ERα binding activity IC_{50} (nM)	Generation	Chemical	MG-MID (μM)	ERα binding activity IC_{50} (nM)	Generation
17 β -estradiol	-	1.89		RID-SG2	15.50	>10000	2nd
4-Hydroxytamoxifen	5.50	2.72		RID-SG3	6.46	>10000	2nd
Tamoxifen	7.41	21.1		RID-SG4	26.3	>10000	2nd
RID-A	1.48	171	1st	RID-SG7	17.4	>10000	2nd
RID-B	1.17	52.4	1st	RID-SG8	1.55	2910	2nd
RID-C	3.47	295	1st	RID-SG9	1.82	8570	2nd
RID-D	14.8	47.7	1st	RID-SG10	1.45	372	2nd
RID-E	1.20	320	1st	RID-SG11	1.55	127	2nd
RID-F	2.45	154	1st	RID-SG12	1.38	23.9	2nd
RID-G	0.85	26.7	1st	RID-SG13	1.32	234	2nd
RID-H	1.05	44.1	1st	RID-SG14	1.51	109	2nd
RID-SB1	1.38	>10000	2nd	RID-SG15	1.51	835	2nd
RID-SB2	5.40	>10000	2nd	RID-SG16	1.10	1230	2nd
RID-SB3	10.5	>10000	2nd	RID-SB22	1.51	383	3rd
RID-SB4	43.7	>10000	2nd	RID-SB23	1.70	253	3rd
RID-SB7	5.77	>10000	2nd	RID-SB17	2.00	380	3rd
RID-SB8	1.82	>10000	2nd	RID-SB24	2.63	568	3rd
RID-SB9	2.69	4640	2nd	RID-SF22	1.79	421	3rd
RID-SB10	1.51	2370	2nd	RID-SF23	6.03	656	3rd
RID-SB11	1.44	582	2nd	RID-SF17	12.0	428	3rd
RID-SB12	1.34	145	2nd	RID-SF24	17.0	1840	3rd
RID-SB13	1.31	218	2nd	RID-SG22	1.05	253	3rd
RID-SB14	1.44	280	2nd	RID-SG23	1.70	387	3rd
RID-SB15	1.62	4880	2nd	RID-SG17	1.78	515	3rd
RID-SB16	1.46	702	2nd	RID-SG24	2.04	1560	3rd
RID-SG1	2.29	>10000	2nd				

Table 2. COMPARE Analysis of RID-SB1 and RID-SB8.

Chemical	Ranking	Drug or inhibitor	r	Function
RID-SB1	1	NVP-AEW541	0.505	IGF-1R inhibitor
	2	Bortezomib	0.481	proteasome inhibitor
	3	RDEA119	0.474	MEK inhibitor
	:	:	:	:
	>100	tamoxifen	0.199	ER antagonist
RID-SB8	1	PB28 dihydrochloride	0.563	sigma-2 receptor agonist
	2	MEK inhibitor I	0.549	MEK inhibitor
	3	Raf1 Kinase Inhibitor I	0.549	Raf1 inhibitor
	:	:	:	:
	>100	tamoxifen	0.314	ER antagonist

[a] The ID for the chemical in JFCR39 database

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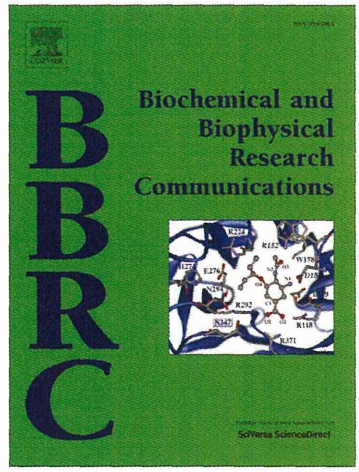

Novel tamoxifen derivative Ridaifen-B induces Bcl-2 independent autophagy without estrogen receptor involvement

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